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# Targeting Mononuclear Phagocytes to Treat COVID-19

*Brandt D. Pence and Theodore J. Cory*

## Abstract

Coronavirus disease 2019 (COVID-19) and its etiological agent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have caused considerable illness and death worldwide. The innate immune system seems to play a principal in the disease, as a hallmark of severe COVID-19 is excessive inflammation. Monocytes and macrophages are important innate immune cells that become pro-inflammatory and promote adaptive immune responses during viral infection. In this chapter we present evidence linking these cells to severity of COVID-19. Namely, monocytes and macrophages infiltrate the infected tissue during the early stages of infection and show pro-inflammatory responses that appear to be linked to those predicting tissue pathology during disease. Additionally, studies in isolated cells demonstrate that monocytes and macrophages respond by producing pro-inflammatory cytokines when directly stimulated by SARS-CoV-2. While most anti-inflammatory pharmaceutical treatments for COVID-19 have focused on systemic infiltration, some of the most promising have known or suspected effects on monocyte and macrophage inflammatory responses. Therefore, targeting these cells to treat severe COVID-19 is a promising strategy for this important disease.

**Keywords:** COVID-19, SARS-CoV-2, monocytes, macrophages, innate immunity

## 1. Introduction

A novel highly pathogenic coronavirus emerged in Hubei Province, China in the latter months of 2019. The government of the People's Republic of China informed the World Health Organization of the outbreak of the virus, which was preliminarily named novel coronavirus 2019 (2019-nCov), on 31 December 2019. Early observational studies found high incidences of fever, cough, and fatigue in hospitalized patients with diagnosed infection, and pneumonia, acute respiratory distress syndrome, and higher plasma pro-inflammatory cytokine levels were also common in those admitted to the Intensive Care Unit [1]. Comorbidities including older age and diabetes were found to be associated with worse outcomes [2].

Patient samples were utilized to isolate a betacoronavirus which was distinct from previous highly pathogenic coronaviruses such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which caused epidemic outbreaks in 2002–2003 [3] and 2012 [4] respectively. Molecular epidemiology studies on 2019-nCov found that the new virus shared approximately 80% sequence identity to SARS-CoV, and further that it shared ~96% sequence identity to the known bat coronavirus RaTG13 [5], suggesting a potential bat origin. Later papers have suggested transmission to

humans through an intermediate host, with leading candidates including Malayan pangolins and minks [6], but to date no natural viral reservoir or intermediate host has been found despite extensive surveys. Although there has been some speculation as to a laboratory origin of 2019-nCov, this is generally rejected by most in the scientific community [7–10], and to date there is no compelling evidence of a human origin for the virus.

In March 2020, the World Health Organization declared a worldwide pandemic of Coronavirus Disease-2019 (COVID-19), the disease of which 2019-nCov is the etiological agent. Likewise in that month, the International Committee on Taxonomy of Viruses (ICTV) officially named the new virus the Severe Acute Respiratory Coronavirus-2 (SARS-CoV-2) [11], and this nomenclature will be utilized throughout the rest of the chapter. To date, SARS-CoV-2 and COVID-19 have caused tremendous morbidity and mortality worldwide, with deaths from COVID-19 numbering more than 3 million as of mid-April 2021 [12].

## **1.1 Clinical indicators of COVID-19**

COVID-19 generally presents with some combination of cough, fever, and/or dyspnea, with less prevalent symptoms including diarrhea, myalgia, and nausea/vomiting [13]. Clinical immunology indicators of severe COVID-19 also include lymphopenia and thrombocytopenia [13] as well as significant elevations of circulating pro-inflammatory cytokines and other inflammation markers such as C-reactive protein [14]. Chest CT scans detect ground glass opacities and pneumonia in a substantial fraction of COVID-19 patients with severe disease [13, 15]. Severe COVID-19 can progress to acute respiratory distress syndrome [16] and can lead to death.

Multiple co-morbidities are associated with severity of COVID-19. Among these, advanced age is the strongest predictor of morbidity and mortality [17], and increased disease severity is also linked with pre-existing diabetes [18] and severe asthma [17]. In addition to the pulmonary system, SARS-CoV-2 has been found to infect other organ systems including the cardiovascular, central nervous, and gastrointestinal tract systems [19], therefore a variety of other symptoms can occur during COVID-19. A subset of COVID-19 patients develop persistent symptoms which last for weeks or months, which has been described as post-acute COVID-19 syndrome or colloquially as “long COVID” [20]. The etiology of long COVID is unknown, but does not appear to be associated with persistence of replication-competent SARS-CoV-2 [21].

## **2. Overview of the immune system**

### **2.1 Adaptive immune system**

The vertebrate immune system is generally divided into two complementary arms. The adaptive immune system is highly specialized and recognizes specific protein motifs corresponding to individual pathogens. Adaptive immunity is primarily mediated by T and B lymphocytes, with T cells further divided into cytotoxic T cells (CD8+) and several classes of helper T cells (CD4+). Cytotoxic T cells recognize infected cells through interactions of the T cell receptor (TCR) with antigen-bound class I major histocompatibility complex (MHC) molecules, and kill these cells via release of cytotoxins such as perforins and granzymes. Likewise, helper T cells recognize pathogens through interactions of their TCR with antigen-bound class II MHC molecules on antigen presenting cells, and serve to instruct activation

of B cells or CD8<sup>+</sup> T cells through the release of various cytokines. Finally, B cells produce pathogen-specific antibodies, which bind extracellular pathogens to allow them to be neutralized by a variety of methods. Adaptive immune responses are highly important during SARS-CoV-2 infection and are key to successful clearance of the virus from the host. However, these aspects of immunity during COVID-19 are otherwise beyond the scope of this chapter, and we can point readers to a recent review article on this subject [22].

## 2.2 Innate immune system

Unlike adaptive immunity, innate immunity is not specific to individual pathogens, but is instead a host system which allows for conserved responses to broad classes of pathogens. A key facet of the innate immune response is inflammation, which allows for the destruction and removal of infected or damaged cells, as well as tissue cleanup and infiltration of additional immune effector cells to the site of infection. A key component of innate immunity is inflammation. Chemokines and pro-inflammatory cytokines respectively attract innate immune cells to the site of infection and activate them, thereby promoting their various effector functions. Neutrophils are early responder cells which perform phagocytosis of pathogens and kill microorganisms via the release of soluble anti-microbial molecules and neutrophil extracellular traps. Dendritic cells take up pathogens and damaged cell components via phagocytosis and migrate to the lymph nodes, where they present antigen on class II MHC to activate CD4<sup>+</sup> T cells. Likewise, macrophages can perform similar antigen presenting functions, and are also key orchestrators of the immune response via the release of cytokines and chemokines. A variety of other cell types are active during an innate immune response to pathogens such as SARS-CoV-2, and we point readers to a recent review on this topic [23].

## 2.3 Monocytes

The remainder of this chapter will principally focus on the contributions of monocytes and macrophages to COVID-19. These are innate immune cells of myeloid lineage. Monocytes arise in the bone marrow from common myeloid progenitor cells, which are also the precursor to erythrocytes, mast cells, and neutrophils among other cell types [24]. Monocytes circulate in the bloodstream and perform important innate immune effector functions, including antigen presentation, phagocytosis, and immune signaling through cytokine production [25]. Monocytes recognize broad classes of pathogen (e.g., gram-negative bacteria, dsRNA viruses, etc.) via pathogen binding to cell surface and intracellular pattern recognition receptors (PRRs) and respond by phagocytosis and/or cytokine production to further orchestrate the immune response [25].

In humans, three traditional monocyte phenotypes are widely recognized: classical (CD14<sup>+</sup>CD16<sup>-</sup>), intermediate (CD14<sup>+</sup>CD16<sup>+</sup>), and non-classical (CD14<sup>dim</sup>CD16<sup>+</sup>) [26]. Classical monocytes make up the bulk of circulating monocytes (>80%) and are highly phagocytic and potent cytokine producers. Intermediate monocytes are enriched for antigen presenting MHC molecules and produce cytokines under PRR binding, and non-classical monocytes have patrolling and wound healing functions and are less responsive to PRR binding compared to classical monocytes [27]. However, intermediate and non-classical monocytes are also associated with increased basal inflammation and are increased in circulation in a number of chronic diseases [27], thereby suggesting that proportional increases in CD16<sup>+</sup> monocyte populations may be reflective of detrimental innate immune responses.



## 2.4 Macrophages

Macrophages are tissue mononuclear phagocytes that participate in innate immune defense and stimulation of the adaptive immune system. During an inflammatory event, peripheral monocytes invade tissue and differentiate to macrophages to carry out host defense, tissue remodeling, and cellular signaling activities. Tissue enrichment of monocyte-derived macrophages is therefore a hallmark of many infections and contributes to immunopathology during acute disease [28–30]. Additionally, many long-lived tissue resident macrophages are not monocytic in origin and instead arise during embryonic development [31]. Tissue resident macrophages often have distinct nomenclature (e.g. Kupffer cells – liver; microglia – brain; osteoclasts – bone) and can perform highly diverse functions depending on tissue environment.

Macrophages are phenotypically heterogeneous and can be polarized along the inflammatory spectrum to mediate diverse functions which are pro-inflammatory (e.g., T cell stimulation) or anti-inflammatory (e.g., wound healing) in nature [32]. Macrophages are often classified as M1/pro-inflammatory or M2/anti-inflammatory depending on their polarization signals and their expression of cell surface or intracellular markers, which is recognized to be an oversimplification but persists due to the utility of studying these phenotypes, especially *in vitro*.

Within the tissue, macrophages play principal roles in phagocytosis of dead cells, cell debris, and extracellular pathogens, and additionally present antigens to CD4<sup>+</sup> T cells to activate adaptive immune responses [33]. Macrophages also recognize pathogens through PRR binding and produce cytokines and chemokines to orchestrate the immune response, including both initiation and resolution of inflammation, the latter of which is key to successful tissue repair.

## 3. Monocytes and macrophages in COVID-19

A substantial body of evidence now demonstrates the importance of immune function during COVID-19 [22, 23]. Here we will focus on the potential contributions of monocytes and macrophages to severe COVID-19, especially as it pertains to the hyperinflammatory environment characteristic of severe disease [34–36]. A large number of cytokines have been shown to be upregulated during COVID-19 and associated with poor outcomes, including interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-10, IL-17A, IL-33, monocyte chemoattractant protein-1 (MCP-1), granulocyte colony stimulating factor (G-CSF), interferon (IFN)- $\gamma$ , IFN $\gamma$ -inducible protein (IP10), and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) among others [37–43].

### 3.1 Monocyte infiltration into infected tissue

The infiltration of monocytes and subsequent increase in monocyte-derived macrophages is a hallmark of infection severity for a number of viruses [28–30]. Several observations have now suggested that this is also the case in COVID-19. A variety of single cell RNA sequencing studies have noted increases in monocytes and macrophages in collected bronchoalveolar lavage fluid that are associated with severe disease [44–46]. Notably, infiltrating monocyte-derived macrophages seem ultimately to be the principal contributors to disease severity, rather than resident alveolar macrophages [45, 47, 48]. Likewise, several post-mortem analyses of patients who died due to COVID-19 have found significant numbers of monocytes and monocyte-derived macrophages in tissues, especially lungs [49–52].

A key drawback to observational human studies is that they cannot empirically determine whether monocyte infiltration and macrophage increases are due to SARS-CoV-2 infection itself, due to the ethical issues with infecting human subjects with the virus itself in a prospective manner. Animal studies are often used in such instances, and this is complicated in COVID-19 research by the general non-susceptibility of traditional laboratory mouse models to infection by SARS-CoV-2. This is due to significant sequence differences between mouse and human angiotensin converting enzyme 2 (ACE2), the principal receptor for SARS-CoV-2 [53].

Nevertheless, while standard mouse models are not susceptible to viral infection, a large number of other laboratory animals have been established as models of COVID-19. Experimental infection in these animals invariably leads to monocyte and macrophage infiltration into pulmonary tissue, as has been shown to date with human ACE2-expressing transgenic mice [54, 55], Syrian hamsters [56], rhesus macaques [57–59], and African green monkeys [60] among others. Given the associations with disease shown in human sequencing and pathology studies, as well as the empirical evidence from animal studies demonstrating monocyte/macrophage infiltration during infection, it appears clear that these cells are responding to localized tissue infection during COVID-19. However, the functions being mediated by monocytes and macrophages during the course of SARS-CoV-2 infection are not yet entirely clear.

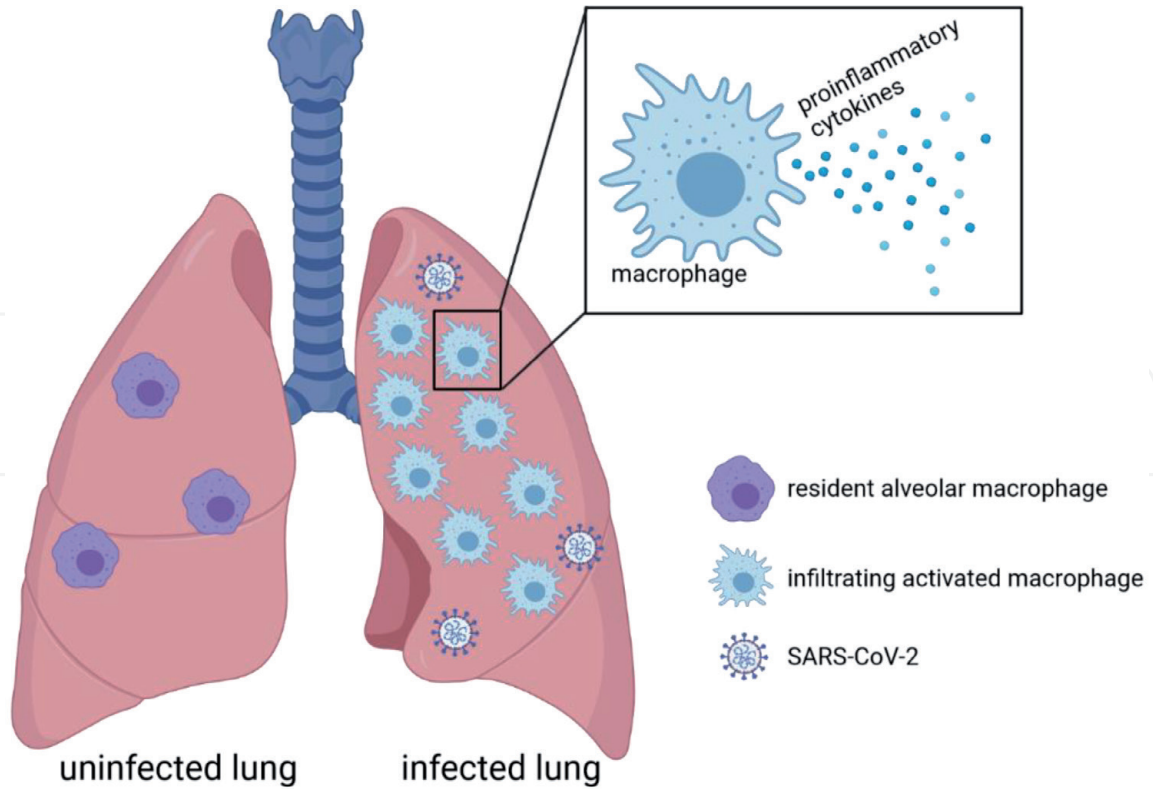
### 3.2 Phenotypic shifts and hyperinflammation

In addition to infiltration into infected tissues, various observational studies have shown shifts in monocyte and macrophage phenotypes towards hyperinflammatory states in COVID-19. One of the most consistent changes noted in COVID-19 immune profiling studies is a decrease in monocyte expression of human leukocyte antigen (HLA)-DR, an MHC class II protein. HLA-DR downregulation has been seen in other inflammatory conditions such as sepsis and is linked to disease severity [61]. In COVID-19, HLA-DR downregulation is a prominent feature [38, 45, 62–66], and could signify immune exhaustion in virus-stimulated cells that could lead to impaired inflammation and antigen presentation, and therefore to defects in adaptive immune responses.

Likewise, both monocytes [67–70] and lung macrophages [44, 45, 71] produce increased levels of pro-inflammatory cytokines during acute SARS-CoV-2 infection as noted in human observational studies. Pro-inflammatory macrophage responses have also been noted in the lungs of experimentally infected non-human primates [60, 72, 73], thereby linking macrophage inflammation to infection with SARS-CoV-2 itself (and not some other biological factor). Therefore, it appears that SARS-CoV-2 infection causes inflammatory responses in macrophages and monocytes, although the immediate proximal cause of this response cannot be identified solely via immune cell phenotype profiling.

### 3.3 Responses to direct infection

Both macrophage infiltration to infected tissues and resulting hyperinflammation in these cells can be explained by indirect mechanisms (e.g., infected cells activating monocytes/macrophages via cytokine signaling). However, several *in vitro* studies have demonstrated that monocytes and macrophages react to infection with SARS-CoV-2 by mounting pronounced inflammatory responses [74–76]. It is likely that infection of these cells is abortive (i.e., does not produce additional infectious virus) [74, 75, 77], so these responses may be mediated by viral protein binding to cellular receptors rather than by recognition of replicating viral RNA.



**Figure 1.** Schematic of lung macrophage populations during COVID-19. Uninfected lungs (left) maintain a resident population of alveolar macrophages. During SARS-CoV-2 infection (right), infiltrating monocyte-derived macrophages become activated and produce pro-inflammatory cytokines. Created with BioRender.com.

Additionally, immunometabolic activation has been demonstrated in infected monocytes, which upregulate glycolytic activation [78] and accumulate intracellular lipid droplets [79] when exposed to SARS-CoV-2. Metabolic reprogramming is a hallmark of innate immune activation [80], and thus may be a mechanism by which myeloid cells mount their initial inflammatory responses to SARS-CoV-2. A schematic of lung macrophage populations with and without SARS-CoV-2 infection is shown in **Figure 1**.

#### 4. Targeting monocytes and macrophages

Targeting monocytes and macrophages as a strategy to improve outcomes in people infected with SARS-CoV-2 has been an area of considerable interest since the beginning of the COVID-19 pandemic. As key effectors of the innate immune system, they play an important role orchestrating the immune response to SARS-CoV-2. This approach has been primarily focused on systemic anti-inflammatories, although a number of other strategies are under investigation. While a variety of small molecule and biologic based strategies have been investigated to treat COVID-19, there has been a relative dearth of strategies which have been shown to improve disease outcomes.

##### 4.1 Dexamethasone

One such drug which has been recommended for use in critically ill COVID-19 subjects is the corticosteroid dexamethasone. A number of trials, most notably the RECOVERY trial have investigated the use of dexamethasone in individuals receiving advanced care for COVID-19, and have shown that the drug may of therapeutic



utility for severe cases [81]. This trial showed that in ventilated patients and patients receiving supplemental oxygen, that administration of IV dexamethasone resulted in a significant increase in 28-day mortality. Interestingly, however, the same results were not observed for individuals not receiving supplemental oxygen.

Among other mechanisms, one way by which corticosteroids decrease inflammation is by modulating cytokine release from monocytes and macrophages [82]. This can include, among other cytokines, IL-8, GM-CSF, and TNF- $\alpha$  [82, 83]. By modulating cytokine production in monocytes and macrophages, dexamethasone may lessen the strong cytokine storm that occurs in many people infected with SARS-CoV-2 [84].

#### 4.2 Baricitinib

Baricitinib is a JAK 1 and 2 inhibitor which has currently received an emergency use authorization to be used in combination with remdesivir for the treatment of COVID-19. Previously, it had been approved for the treatment of rheumatoid arthritis [85]. In patients with COVID-19, in combination with remdesivir it has been shown to be superior to remdesivir alone in improving clinical status, especially in ventilated individuals [86]. Baricitinib likely functions in COVID-19 by decreasing the release of inflammatory cytokines from immune cells, including macrophages.

Non-human primate studies of baricitinib has shown that it can decrease the production of pro-inflammatory cytokines in lung macrophages, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$  [72]. These modulations in cytokine expression from macrophages also blunted neutrophil influx into the lungs of these animals, which likely represents the mechanism by which the drug improves COVID-19 outcomes.

#### 4.3 Tocilizumab

Tocilizumab is a monoclonal antibody therapeutic which has been approved for the treatment of rheumatoid arthritis [87]. Its mechanism of action is to act as an antagonist for the interleukin-6 receptor. By blocking this receptor, it is able to decrease signal transduction of this pathway and decrease the host inflammatory response. During the COVID-19 pandemic, it has gained considerable interest as a therapeutic for COVID-19. Its use is recommended as a single IV dose in combination with dexamethasone in patients who are critically ill in the ICU and receiving mechanical ventilation [88]. The evidence supporting the use of tocilizumab in COVID-19 is somewhat mixed, with some studies showing no benefit in the disease [89].

Macrophages are key producers of IL-6, and the IL-6 receptor is expressed on the surface of macrophages [90]. In COVID-19, some patients experience an overly strong cytokine response, commonly referred to as a cytokine storm, or hyperinflammation. Treatment with tocilizumab may be able to decrease this strong inflammatory response through blunting IL-6 signaling [91].

#### 4.4 Non-steroidal anti-inflammatory drugs (NSAIDs)

To date, there has been considerable controversy to the potential benefit of NSAIDs for the treatment of COVID-19. These drugs inhibit the activity of the cyclooxygenase isoforms 1 and 2 [92]. In March of 2020, the French Minister of Health raised concerns based on case reports in the country showing individuals with worsened symptoms after the administration of NSAIDs [93]. This was further supported by previous studies in lower respiratory infections suggesting



that NSAID usage could worsen disease outcomes. These studies, however, were relatively weak, and additional research is likely necessary to determine the effect of these drugs on respiratory infections [94]. There is some evidence that suggests that NSAIDs may be able to decrease the production of pro-inflammatory cytokines including TNF- $\alpha$  from macrophages, which may represent a potential mechanism of action of any potential benefits for the treatment of COVID-19 [95].

5. Conclusions

COVID-19 is an emergent and ongoing disease with substantial public health and sociological implications. It is clear that inflammation underlies severe COVID-19, and so the biological mechanisms by which this occurs are of substantial interest. Monocytes and macrophages are important innate immune cells that appear to play central roles in COVID-19, as they infiltrate infected tissues and produce pro-inflammatory cytokines during infection. Some current biologic and non-steroidal anti-inflammatory therapies which may be efficacious in treating COVID-19 have known effects on macrophages and monocytes. However, these have primarily been used systemically, so the utility of directly targeting mononuclear phagocytes as a therapeutic for COVID-19 remains in need of investigation. A brief summary of evidence for anti-inflammatory drugs used to treat COVID-19 is presented in **Table 1** below.

Future studies are needed to define the molecular mechanisms by which monocytes and macrophages respond to SARS-CoV-2, either due to direct infection or due to signaling from nearby infected cells. A fuller understanding of how myeloid cells become activated during COVID-19 will allow for more targeted therapies to be developed. These may be more efficacious than the current systemic anti-inflammatory treatments outlined in Section 4 above, as they would represent evidence-based strategies for treating hyperinflammation during severe COVID-19.

Treatment	Proposed mechanism	Evidence	References
Corticosteroids	Decreased cytokine release	Weak	[81]
Baricitinib	JAK inhibitor	Modest	[72, 86]
Tocilizumab	IL-6R inhibitor	Mixed	[88, 89]
NSAIDs	Unclear for COVID-19	Weak	[93]

**Table 1.**  
*Targeting COVID-19 with anti-inflammatory drugs.*

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## Author details


Brandt D. Pence<sup>1\*</sup> and Theodore J. Cory<sup>2</sup>

1 College of Health Sciences, University of Memphis, Memphis, TN, USA

2 Department of Clinical Pharmacy and Translational Science, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN, USA

\*Address all correspondence to: [bdpence@memphis.edu](mailto:bdpence@memphis.edu)

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'Biotechnology to Combat COVID-19' is a collaborative project  
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